Exhibit 4a #23

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A live *Pasteurella haemolytica* vaccine efficacy trial

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SUMMARY

A live *Pasteurella haemolytica* serotype 1 vaccine was used in an efficacy trial conducted on 100 lightweight feeder calves purchased from a Florida ranch. Forty-one calves were inoculated with the vaccine intradermally in the neck. Fifty-nine calves served as nonvaccinated controls. Fourteen days later, the calves were shipped to an order buyer in eastern Tennessee, where the calves were mixed with 60 local calves in a community sale barn for 72 hours. After 3 additional days, the calves were shipped to a research feedlot in Bushland, Tex. They remained in the feedlot for 56 days, and the test was concluded 76 days after vaccination.

The *P haemolytica* vaccine had no significant effect on performance, morbidity, or mortality. There was no significant difference between the vaccinated and nonvaccinated calves in the number of times *Pasteurella* was isolated. The calves became seropositive to bovine viral diarrhea virus, respiratory syncytial virus, and infectious bovine rhinotracheitis (IBR) virus during the 76-day experiment. All calves initially were seropositve to parainfluenza-3 virus. A virulent outbreak of IBR occurred 30 days after the calves arrived at the feedlot. Before the onset of IBR, the isolation of *P haemolytica* serotype 1 from nasal turbinates was rare (2 of 500 nasal swabs). After the IBR outbreak, *P haemolytica* serotype 1 was isolated from 40 of 92 calves.

BOVINE RESPIRATORY DISEASE (BRD) of lightweight (136 to 250 kg) feeder calves is a marketing/management-related disease that has severe negative economic impact on the feeder calf industry.[1] Unfortunately, the greatest economic losses are sustained in the feedyards, which have little control over prior management practices that could reduce the prevalence of BRD. A large supply of feeder calves is derived from southeastern United States, where cow herds frequently do not exceed 12 in number and the marketing of 2 or 3 calves at one time is a common practice. Thus, it takes many cow-calf operations to provide 100 or more lightweight feeder calves to make a truckload. The various management practices of these cow-calf operations have great potential to influence the prevalence of BRD, which usually develops 7 to 14 days after the calves arrive at feedyards in the High Plains.[2] Bovine respiratory disease is recognized to be of complex causation[3]; however, *Pasteurella haemolytica* serotype 1 frequently is incriminated as the bacterium that causes the death of the calf.[2,a]

The control of *P haemolytica* would diminish its deleterious effect on animal health and performance and would be a gain for the feeder calf industry. Thus far, however, *P haemolytica* bacterin is not widely recognized as efficacious.[3,4] Proving efficacy of a *P haemolytica* bacterin to reduce or prevent BRD is further complicated by the inability of research workers to reproduce the disease.[5]

The purpose of this report is to evaluate the efficacy of a commercially available, live *P haemolytica* vaccine[b] under semicontrolled conditions.

Materials and Methods

Calves—One-hundred Brahman-type steers weighing an average of 126 kg were purchased from an Indian reservation ranch in Florida.

Movement and processing of calves—The calves were trucked 62 km from the ranch of origin to an Ocala, Fla,

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[a]Shewen PE, Wilkie BN. Immunity to *Pasteurella haemolytica* serotype 1 in bovine respiratory disease (abstr), in Loan RW (ed), in *Proceedings*. N Am Symp on Bovine Respiratory Disease. Texas A&M University Press, College Station, Tex 1983;480–481.

[b]PRECON-PH, AH Robins Co, Richmond, Va.

ranch that had processing facilities. On arrival, the calves were placed on pasture. Three days later, each calf was weighed and processed. Processing consisted of (1) vaccination against *Clostridium chauvoei, C novyi, C septicum*, and *C sordellii*[c]; (2) identification with ear tags; (3) blood sampling by jugular venipuncture; (4) obtaining nasal turbinate swab specimens; and (5) vaccination against *P haemolytica*. The calves remained on this ranch for 14 days and were observed daily. They were placed in a pasture, fed alfalfa hay, commercial concentrate mix, salt, and trace mineral block ad libitum. The calves then were shipped by truck 800 miles to an order buyer in eastern Tennessee, where blood samples and nasal swab specimens were obtained again. The cattle were mixed for 3 days with 60 local sale-barn calves of similar weight. The calves were provided with water, alfalfa hay, and a commercial concentrate ad libitum. The calves remained at the order buyer barn for 6 days, then were trucked to a research feedlot located at Bushland, Tex.

On arrival at the feedlot, the calves were placed in 4 open pens, fed 60% concentrate diet and alfalfa hay, watered, and allowed to rest overnight. The following day, each calf was weighed and processed. Processing consisted of (1) revaccination against *C chauvoei, C novyi, C septicum*, and *C sordellii*[c]; (2) deworming with thiabendazole paste; (3) injection of vitamins A and D (1 million IU and 150,000 IU, respectively); (4) application of pour-on insecticide; (5) replacing lost eartags; (6) blood sampling by jugular venipuncture; (7) recording rectal temperature; (8) collecting nasal turbinate swab specimens; and (9) starting sick calves on a 3-day course of spectinomycin (15 mg/kg of body weight).

Vaccine—A live, freeze-dried *P haemolytica* vaccine was reconstituted with diluent provided. A dose of 0.5 ml was inoculated intradermally into the neck of 41 calves. Nine calves were inoculated similarly with dilent only, and the remaining 50 calves were left uninoculated.

Scoring and treatment of calves for sickness—The calves were observed at dawn for signs of disease. A point system was used, with one point being recorded for each of the following: ocular discharge, nasal discharge, gaunt appearance, or depression. If a calf was assigned 2 points, it was isolated and its rectal temperature was recorded. If the temperature exceeded 40 C, 2 more points were assigned. A calf with 4 points or more was considered sick, and antibiotic therapy was instituted. The calf was confined to a "sick pen" for at least a 3-day course of therapy. Body weights and rectal temperatures of sick calves were recorded daily. If the calf recovered after 3 days, it was observed one additional day; if it was again clinically normal, it was returned to its original holding pen. If, after 3 days, rectal temperature still exceeded 40 C, another antibiotic was used for 3 days.

Specimen collecting and weighing calves—Each calf was weighed each week. Nasal turbinate swab specimens and blood samples were collected weekly.

Dead calves were submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory at Amarillo, Tex, to determine the cause of death. Nasal turbinate swab specimens were submitted to the National Animal Disease Center, Ames, Iowa, for *Pasteurella* isolations and typing. Swabs for *Mycoplasma* isolations were submitted to The Texas A&M University Agriculture and Extension Center, San Angelo, Tex. Swabs for *Pasteurella* isolations were placed in screw-cap test tubes and placed on dry ice or cooled with ice and stored at −85 C. Swabs for *Mycoplasma* isolation were washed in screw-cap vials each containing 2 ml of modified Hayflicks medium with 10% horse serum and immediately were placed on dry ice or cooled with ice, frozen, and stored at −85 C. All nasal swabs in their containers were shipped with dry ice.

Statistical calculations—The data were analyzed by chi-square and analysis of variance as a completely randomized design. Treatment differences were considered significant at the $P < 0.05$ level.

[c]Convac CSNS Affiliated Lab, Bristol, Tenn.

Results

There were no significant differences in morbidity, mortality, and performance between the *P haemolytica*-vaccinated and the nonvaccinated calves (vaccinates—morbidity 83%, mortality 4.9%; nonvaccinates—morbidity 88%, mortality 10.2%).

Eight calves (2 vaccinates and 6 nonvaccinate controls) died of pneumonia typical of the BRD complex. *Pasteurella haemolytica* serotype 1 was isolated from the lungs of 1 vaccinated calf and 3 nonvaccinated calves. *Pasteurella multocida* was isolated from the lungs of 3 nonvaccinated calves. A virulent outbreak of infectious bovine rhinotracheitis (IBR) occurred 30 days after the calves arrived at the feedlot. The IBR virus was isolated from several tissues from one calf at time of necropsy, 40 days after arriving in the feedlot. This calf was the first one to die after the appearance of clinical IBR in the group of calves. Five calves died of BRD between the 40th and 53rd days in the feedlot. All 5 calves had tracheal lesions consistent with those seen after virulent IBR virus infection.

Daily weight gains (mean, 951 g), feed dry matter intake (4.3 kg/day), and gain:feed ratio (221 g gain:kg feed dry matter) of vaccinated and nonvaccinated calves were similar during the 56 days in the feedlot.

The frequency of isolation of *Pasteurella, Mycoplasma*, and *Ureaplasma* from nasal swab specimens in relationship to the time of marketing is reported in Table 1. *Pasteurella multocida* was found in 55 calves (24 vaccinates and 31 nonvaccinates) before vaccination and in 31 calves (14 vaccinates and 17 nonvaccinates) at the end of the experiment. Before the IBR outbreak, *P haemolytica* serotype 1 was found in only one nonvaccinated calf during the first 28 days of the experiment (8 days after arrival at the

TABLE 1—Frequency of isolation of *Pasteurella, Mycoplasma*, and *Ureaplasma*

	Isolation of organisms according to place and time on test				
	Ph1	Ph2	Pm	M	U
			Ranch (day 0)		
Vaccinates	0/41 (0)*	20/41 (49)	24/41 (59)	18/30 (60)	11/19 (58)
Nonvaccinates	0/59 (0)	23/59 (39)	31/59 (53)	18/38 (47)	1/25 (4)
			Order buyer (day 14)		
Vaccinates	0/41 (0)	18/41 (44)	20/41 (49)	30/40 (75)	24/35 (69)
Nonvaccinates	0/59 (0)	18/59 (31)	31/59 (53)	35/53 (66)	34/51 (67)
			Feedlot (day 21)		
Vaccinates	0/40 (0)	7/41 (17)	23/41 (56)	8/17 (49)	9/17 (53)
Nonvaccinates	0/59 (0)	11/59 (19)	39/59 (66)	12/18 (67)	10/18 (56)
			Feedlot (day 76)		
Vaccinates	15/39 (38)	6/39 (15)	14/29 (26)	36/36 (100)	29/35 (83)
Nonvaccinates	25/53 (47)	2/53 (4)	17/53 (32)	50/50 (100)	39/44 (89)

*No. of isolations/No. of calves (%).

Ph1 = *Pasteurella haemolytica* serotype 1; Ph2 = *P haemolytica* serotype 2; Pm = *P multocida*; M = *Mycoplasma* sp; U = *Ureaplasma* sp.

feedlot). The frequency of isolation of *P haemolytica* serotype 1 increased dramatically following the IBR outbreak; 40 isolates were recovered on the 76th day of the experiment. Fifteen of these isolates were from vaccinates, and 25 were from nonvaccinates.

Mycoplasma sp was isolated from 36 calves (18 vaccinates and 18 nonvaccinates) at the beginning of the experiment. At the completion of the experiment, *Mycoplasma* sp was isolated from 86 calves (36 vaccinates and 50 nonvaccinates). *Ureaplasma* sp was isolated from 12 calves (11 vaccinates and 1 nonvaccinate) before vaccination and from 68 calves (29 vaccinates and 39 nonvaccinates) at the completion of the experiment.

Serologic screens for IBR, bovine viral diarrhea (BVD), respiratory syncytial virus, and parainfluenza-3 virus were compared. All calves were seropositive to parainfluenza-3 virus at the start of the experiment. Seroconversion for IBR virus, 56% (22/39 samples); BVD virus, 68% (63/92 samples); and respiratory syncytial virus, 100% (36/36) occurred before day 53 of the experiment.

Discussion

A seromucous nasal discharge was noticed in many of the calves before vaccination in Florida, and a similar discharge continued in many of the calves throughout the 76-day study, both in vaccinates and nonvaccinates. Clinically, the calves were not affected adversely by this discharge. The discharge was attributed to *P multocida* (55 isolates before vaccination) and possibly in part to *P haemolytica* serotype 2 (43 isolates before vaccination) isolated before vaccination. On day 76, *P multocida* was isolated from 31 calves; *P haemolytica* serotype 2 was isolated from 8 calves. It was concluded that the *P multocida* contributed more to the chronic nasal drainage than did the *P haemolytica* serotype 2, based on the number of isolates recovered and the lack of progression of the clinical condition throughout the study.

Normally, most morbidity and mortality occurs within the first 2 weeks at the Bushland, Tex, research feedlot; however, 13 calves were either retreated or died after their first 2 weeks in the feedlot. This mortality and morbidity followed an IBR outbreak in the calves.[6,7] The *P haemolytica* serotype 1 was recovered from 2 of 500 nasal swabs before the IBR outbreak. However, 40 isolations of *P haemolytica* serotype 1 (15 from vaccinates and 25 from nonvaccinates) were made at the end of the experiment. It is not known whether *P haemolytica* serotype 1 was reintroduced by the 2 carrier calves or whether the IBR infection induced the bacteria to emerge from an endogenous source. Regardless, the *Pasteurella* vaccine appeared to have little or no influence on the recovery of *P haemolytica* serotype 1 from vaccinates and nonvaccinates throughout the study.

The frequency of isolation of *Mycoplasma* and *Ureaplasma* increased from the beginning to the end of the study as follows: beginning—36 *Mycoplasma* isolates from 68 specimens and 12 *Ureaplasma* isolates from 44 specimens; end—86 *Mycoplasma* isolates from 86 specimens and 68 *Ureaplasma* isolates from 79 specimens.

Mortality was 5% in the vaccinates and 10% in the nonvaccinates. This difference was not significant, but the trend was in favor of some protection from the vaccine. There was essentially no difference in morbidity between the 2 groups.

Particular care was taken to see that antibiotics were not used during the early phase of the experiment, when the vaccine bacteria presumably would be growing. There was no evidence that the vaccinated calves excreted *P haemolytica* serotype 1 to nonvaccinated controls.

The vaccine did not improve performance of calves over a period of 76 days. Thus, managers of stocker-feeder operations would not have gained economic advantage from the procedure. Certainly, it would not have been to the operations' advantage to pay a premium for the vaccinated calves in this study.

The calves were minimally stressed before vaccination, and adequate time was allowed for immune reactions before entry of the calves into the market system. Unfortunately, the feeder calf marketing system has evolved because of marketing needs, with little regard for the disease potential it creates. The first practical time that most southeastern calves can be vaccinated after they enter the market system is at the order buyer facility or possibly the auction barn. However, the cost of vaccination would have to be offset by improved performance and reduced morbidity and mortality in the feedlot.

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